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**14. ABSTRACT**

Individuals with germline mutations in BRCA1 have an elevated but incomplete risk of developing ovarian cancer suggesting the presence of genetic modifiers of ovarian cancer in this population. A genome wide association study (GWAS) for ovarian cancer in BRCA1 mutation carriers was initiated in an effort to identify common genetic variants that modify ovarian cancer risk. The discovery phase of the study has been completed. A replication phase of the 6,000 most significantly associated variants is underway. In a separate study, variants in a 19p13.1 locus have been identified as modifiers of both breast and ovarian cancer in BRCA1 and BRCA2 mutation carriers.

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BRCA1, ovarian cancer, genome wide association study, association study

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**Introduction:**

Inactivating mutations in the *BRCA1* tumor suppressor gene have been detected in approximately 10% of all ovarian cancers. Individuals with germline mutations in *BRCA1* have a substantially increased risk of developing ovarian cancer as compared to the general population, with an estimated cumulative risk of ovarian cancer by age 70 of 39% (1). These findings indicate that although *BRCA1* mutation carriers are at high risk for developing ovarian cancer, a sizeable proportion of women who carry a deleterious mutation will not develop this disease. In addition, the findings show that there is considerable variation in the age of onset of ovarian cancer in this population. This variable penetrance and age of onset of ovarian cancer suggest that there are additional genetic and environmental factors that modify the age specific risk of ovarian cancer for *BRCA1* mutation carriers. Common genetic variants that are associated with the risk of ovarian cancer have recently been identified through candidate gene and genome wide association studies in the general population (2-4). This suggests that common genetic variants may also modify ovarian cancer risk in carriers of *BRCA1* mutations. Identification of these genetic risk factors may prove useful for identifying those *BRCA1* carriers at elevated or lowered risk of ovarian cancer compared to the average *BRCA1* carrier. Women at increased risk may subsequently benefit from enhanced screening or certain prevention measures such as prophylactic oophorectomy, whereas women at lowered risk may be able to avoid these types of intervention (5). Thus, we proposed a study aimed at identifying genetic risk factors for ovarian cancer in BRCA1 mutation carriers through a genome wide association study in BRCA1 mutation carriers. The overall intent was to complete a genome wide association study of *BRCA1* carriers, validate candidate risk modifiers, to assess the contribution of these modifiers to sporadic ovarian cancer and to develop risk prediction models.

**Body**

**Aim 1: To conduct a genome-wide association scan in 1,000 *BRCA1* carriers with ovarian cancer and 1,000 age-matched unaffected *BRCA1* carriers.**

Genome Wide Association Study (GWAS)

As described in Preliminary data we conducted a Stage 1 GWAS using Human660W-Quad arrays on 1250 BRCA1 mutation carriers diagnosed with breast cancer and 12500 unaffected. The 1250 unaffected included 361 diagnosed with ovarian cancer. Subsequently we collected and genotyped an additional 434 BRCA1 mutation carriers diagnosed with ovarian cancer on Human660W-Quad arrays. In addition we acquired GWAS genotype data for 120 additional BRCA1 mutation carriers affected with ovarian cancer from collaborators. Together this resulted in GWAS genotype data from 915 BRCA1 mutation carriers diagnosed with ovarian cancer.

Genotyping calls were obtained using the standard Illumina calling algorithm incorporated in the BeadStudio software. As expected gender checks using PLINK software failed to identify male *BRCA1* carriers. Duplicates were identified by identify-by-descent analyses and were removed. Quality control thresholds of >95% variant call rates and >95% sample call rates were applied. Variant with minor allele frequency <0.05 were excluded. In addition single nucleotide polymorphisms (SNPs) displayed divergence from Hardy Weinberg equilibrium $p<1 \times 10^{-7}$ were removed. Final analyses included 897 BRCA1 mutation carriers with ovarian cancer and approximately 540,000 SNPs. Genetic relatedness among samples from different countries and ethnicities can introduce heterogeneity into association studies and cause important SNPs to be overlooked. While this study was restricted to Caucasian BRCA1 carriers the study did include DNA samples from many countries. To account for population stratification, the genotyping data in combination with HapMap data (CEU, Yoruban, Han Chinese populations) on 40,000 SNPs with known phase were analyzed by Eigenstrat. A total of 17 individuals were excluded because of non-caucasian admixture of between 15% and 25%. In collaboration with Drs. Douglas Easton and Antonis Antoniou at the University of Cambridge, we evaluated associations with both breast and ovarian cancer using a retrospective likelihood model. This accounts for the age extremes of affected and unaffected and also applies age related penetrance estimates for *BRCA1* carriers. Carriers were censored at age of onset of disease for those affected with breast or ovarian cancer and age of last follow up or age at

prophylactic mastectomy/oophorectomy for those with no cancer diagnosis. Analyses were adjusted for Country of origin because samples from 26 different centers in 18 countries were included in the study. SNPs used in the study were listed in order of significance of associations with breast cancer and separately for ovarian cancer. For ovarian cancer, no SNPs showed genome wide significance ($p<1 \times 10^{-7}$). However, 10 exhibited associations of $p<1 \times 10^{-5}$ and 37 has associations of $p<1 \times 10^{-4}$. Interestingly, SNPs from two loci (BCN2 and TIPARP) have been found to exhibit genome wide associations with ovarian cancer in the general population (2, 3). When we examined the results from the BRCA1 ovarian cancer GWAS we found that rs1339552 on chromosome 9 in BCN2 ($p=1.9x10^{-5}$) and rs7651446 from TIPARP on chromosome 3 ($p=1.7x10^{-4}$) were highly significantly associated with ovarian cancer. Since the lack of genome wide significance is likely due to the limited number of BRCA1 mutation carriers, these loci can be considered genetic risk factors for ovarian cancer in BRCA1 mutation carriers.

These efforts complete the proposed studies in Aim, which include Task 1-4.

**Aim 2: To further evaluate observed associations between ovarian cancer risk and SNPs implicated in Aim 1 by genotyping 1,500 *BRCA1* ovarian cancer cases and 1,500 unaffected *BRCA1* carriers.**

Validation of Chromosome 19p13.1 associations

Aim 2 of this proposal is focused on validating findings from Stage 1 of the GWAS. Here we describe our efforts to date on this part of the project. Initially we conducted validation studies of candidate SNPs from breast cancer association studies in BRCA1 mutation carriers. The rationale was that because both breast and ovarian cancer occur in BRCA1 mutation carriers, SNPs that modify risk of both breast and ovarian cancer may exist along with SNPs that specifically modify ovarian cancer risk. In our breast studies we further evaluated the 89 most significantly associated SNPs from the breast cancer GWAS in BRCA1 mutation carriers in an additional 5986 *BRCA1* mutation carriers (3012 unaffected, 2974 affected). In the combined analysis of stage 1 and stage 2 samples there was strong evidence of association with breast cancer for five SNPs from a single locus on chromosome 19p13.1 ($P = 2.3\times10^{-9}$ to $3.9\times10^{-7}$). The minor alleles of rs8170 and rs4808611 were associated with an increase in breast cancer risk for *BRCA1* mutation carriers (per allele HR=1.26, 95%CI: 1.17-1.35 for both SNPs) (6). There was no evidence of heterogeneity in the HR estimates for any of the SNPs among the countries of residence. The most strongly associated SNPs are located in a 35kb region containing three genes, *ABHD8* (abhydrolase domain containing 8), *ANKLE1* (ankyrin repeat and LEM domain containing 1), and *C19orf62*. The *C19orf62* gene, which encodes MERIT40 (Mediator of Rap80 Interactions and Targeting 40 kD), is a plausible genetic modifier of breast cancer in *BRCA1* mutation carriers because MERIT40 interacts with BRCA1 in a protein complex that is required for recruitment and retention of the BRCA1/BARD1 ubiquitin ligase at sites of DNA damage.

In this first analysis of <8000 BRCA1 mutation carriers, no association with ovarian cancer was identified for any of the five SNPs from the 19p13.1 locus. However, we subsequently conducted a more complete genotyping study in 12,599 *BRCA1* and 7,132 *BRCA2* mutation carriers, which included 1,465 *BRCA1* mutation carriers and 453 *BRCA2* mutation carriers with ovarian cancer. To assess the influence of these SNPs on ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers we used a competing risk analysis that accounted for the effects on breast and ovarian cancer in parallel. In this competing risk analysis rs67397200 at 19p13.1 was strongly associated with ovarian cancer risk in *BRCA1* (HR=1.16; 95%CI 1.05-1.29; $p=3.8x10^{-4}$) and *BRCA2* (HR=1.30; 95%CI 1.10-1.52; $p=1.8x10^{-3}$) mutation carriers. Similar results were obtained for rs8170 at 19p13.1 (Table 1). Thus, none of the SNPs associated with breast cancer risk in *BRCA1* or *BRCA2* mutation carriers have been associated with ovarian cancer risk. Similarly, none of the SNPs in the BCN2 and TIPARP loci that are associated with ovarian cancer risk in *BRCA1* mutation carriers have been associated with breast cancer risk in *BRCA1* carriers. Furthermore, in the general population, only SNPs in the 8q24 locus are known to influence both breast and ovarian cancer, and these appear to be independent disease-specific effects. Here we report the first variants (from the 19p13.1 locus) found to influence both breast and ovarian cancer risk in either *BRCA1* or *BRCA2* mutation carriers.

**Table 1. Associations with SNPs and breast and ovarian cancer risk using a competing risk analysis model among *BRCA1* and *BRCA2* mutation carriers of European ancestry**

| SNP/ Gene | Alleles | Unaffected N (%) | Breast Cancer N (%) | Ovarian Cancer N (%) | Breast Cancer HR | Breast Cancer 95% CI | Breast Cancer p-value | Ovarian Cancer HR | Ovarian Cancer 95% CI | Ovarian Cancer p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **rs8170 – 19p13.1** | | | | | | | | | | |
| *BRCA1* | GG | 2972 (67.9) | 3730 (63.3) | 923 (66.0) | 1.00 | | | 1.00 | | |
| | AG | 1269 (29.0) | 1936 (32.9) | 434 (31.0) | 1.26 | 1.17 – 1.36 | | 1.23 | 1.08 – 1.42 | |
| | AA | 139 (3.2) | 224 (3.8) | 42 (3.0) | 1.34 | 1.10 – 1.63 | | 1.04 | 0.72 – 1.50 | |
| | per allele | | | | 1.22 | 1.14 – 1.30 | $2.1\times10^{-4}$ | 1.15 | 1.03 – 1.29 | 0.015 |
| *BRCA2* | GG | 1788 (67.0) | 2494 (68.2) | 266 (62.2) | 1.00 | | | 1.00 | | |
| | AG | 796 (29.9) | 1024 (28.0) | 137 (32.0) | 0.95 | 0.85 – 1.05 | | 1.17 | 0.93 – 1.47 | |
| | AA | 83 (3.1) | 138 (3.8) | 25 (5.8) | 1.37 | 1.05 – 1.80 | | 2.72 | 1.65 – 4.48 | |
| | per allele | | | | 1.02 | 0.94 – 1.12 | 0.62 | 1.34 | 1.12 – 1.62 | $1.9\times10^{-3}$ |
| **rs67397200 – 19p13.1** | | | | | | | | | | |
| *BRCA1* | CC | 1903 (51.5) | 2436 (46.0) | 652 (49.7) | 1.00 | | | 1.00 | | |
| | GC | 1498 (40.5) | 2381 (44.9) | 540 (41.2) | 1.28 | 1.18 – 1.38 | | 1.16 | 1.01 – 1.33 | |
| | GG | 298 (8.1) | 484 (9.1) | 120 (9.2) | 1.33 | 1.16 – 1.53 | | 1.36 | 1.07 – 1.73 | |
| | per allele | | | | 1.20 | 1.13 – 1.27 | $4.5\times10^{-10}$ | 1.16 | 1.05 – 1.29 | $3.8\times10^{-4}$ |
| *BRCA2* | CC | 1363 (50.5) | 1866 (50.7) | 194 (45.2) | 1.00 | | | 1.00 | | |
| | GC | 1123 (41.6) | 1489 (40.5) | 184 (42.9) | 0.96 | 0.87 – 1.06 | | 1.15 | 0.92 – 1.44 | |
| | GG | 214 (7.9) | 323 (8.8) | 51 (11.9) | 1.18 | 0.99 – 1.41 | | 1.95 | 1.37 – 2.77 | |
| | per allele | | | | 1.03 | 0.96 – 1.11 | 0.39 | 1.30 | 1.10 – 1.52 | $1.8\times10^{-3}$ |

GWAS validation studies

The original intent for this project was to further evaluate the 384 most significantly associated SNPs from the BRCA1 ovarian cancer GWAS in 3,000 additional BRCA1 mutation carriers including 1,500 with ovarian cancer. However, in 2010 we were provided the opportunity to participate in a large multi-consortium replication study. Specifically, we designed a SNP array (iCOGS) containing 211,000 candidate SNPs from GWAS of various tumor types. A total of 35,000 candidate SNPs were selected from the BRCA1 GWAS including 6,000 from the BRCA1 Ovarian Cancer GWAS. Testing of the arrays showed that 204,000 of the SNPs yielded good quality genotyping. The other 7,000 SNPs were excluded from further consideration.

We proposed to genotype 14,000 DNA samples from BRCA1 mutation carriers on these arrays in contrast to the 3,000 originally planned for Stage 2 of the ovarian cancer GWAS. Investigators in 52 groups from around the world have provided non-amplified genomic DNA samples from approximately 15,000 from female *BRCA1* mutation carriers including approximately 1,400 with ovarian cancer. Each of these samples was evaluated for DNA quality by conducting picogreen and E-gel (Invitrogen) analysis. Samples with low levels of DNA (<250ng available) or with degraded DNA, identified as smearing on Egel analysis, were excluded (n=1,200). A total of 12,700 Caucasian, 150 Malaysian and 204 Hong Kong DNA samples were identified as useful for further validation studies and were subjected to genotyping on the iCOGS array. Genotyping has been ongoing at the Genotype Shared Resource at the Mayo Clinic and is almost complete. Once complete, rigorous quality control measures will be applied as for the GWAS. Associations with breast and ovarian cancer in BRCA1 mutation carriers will be evaluated using the retrospective likelihood model

Based on these efforts we have now completed Tasks 5 to 8 from Aim 2. The next step is to conduct Task 9, which involves analysis of the genotyping data from the iCOGS array.

## Key Research Accomplishments

- Completed a GWAS for ovarian cancer in BRCA1 mutation carriers and identified candidate ovarian cancer risk modifier loci.
- Demonstrated that common variants from a chromosome 19p13.1 locus are associated with ovarian cancer as well as breast cancer risk for BRCA1 and BRCA2 mutation carriers.

## Reportable Outcomes

None

## Conclusion

In summary, we have completed the discovery phase of an ovarian cancer GWAS for BRCA1 mutation carriers, and the genotyping for an extended replication phase of the most significant findings from the study. In an interim analysis of these data we have also successfully identified a 19p13.1 locus that is associated with ovarian cancer risk as well as breast cancer risk for BRCA1 mutation carriers and BRCA2 mutation carriers. Other variants that have been associated with risk of breast cancer in BRCA1 and BRCA2 mutation carriers have shown potential for determination of individual risk of breast cancer in this population. Thus, the 19p13.1 locus that is associated with ovarian cancer risk in BRCA1 carriers and other loci that are expected to be identified through the validation of the GWAS are expected to be useful for improved risk assessment of ovarian and breast cancer risk for BRCA1 and perhaps BRCA2 mutation carriers. In addition, by identifying the underlying causative variants in these loci we expect to develop a greater understanding of the initiation factors involved in breast cancer. For this reason we recommend extending this study to fine mapping efforts of loci associated with ovarian cancer in this population

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## Appendices

None.